Treatment of onychomycosis using a submillisecond 1064-nm neodymium:yttrium-aluminum-garnet laser

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Background: Laser treatment has emerged as a novel treatment modality for onychomycosis.

Objective: We sought to determine thermal response and optical effects of a submillisecond neodymium:yttrium-aluminum-garnet (Nd:YAG) 1064-nm laser on common fungal nail pathogens, and the clinical efficacy and safety of the Nd:YAG 1064-nm laser on onychomycotic toenails.

Methods: A 4-part in vitro and in vivo study was conducted using a Nd:YAG 1064-nm laser. The first portion evaluated 3 different nail pathogens in suspension at 7 heat and time exposures. The second and third parts of the study irradiated pure fungal colonies. The final portion involved an in vivo treatment of toenails over 5 treatment sessions.

Results: A fungicidal effect for *Trichophyton rubrum* was seen at 50°C after 15 minutes, and for *Epidermophyton floccosum* at 50°C after 10 minutes. Limited growth of *Scytalidium* was seen at 55°C after 5 minutes. No inhibition was observed after laser treatment of fungal colonies or suspensions. In vivo treatment of toenails showed no improvement in Onychomycosis Severity Index score.

Limitations: The Nd:YAG 1064-nm laser was the only laser tested.

Conclusions: Laser treatment of onychomycosis was not related to thermal damage or direct laser effects. In vivo treatment did not result in onychomycosis cure. (J Am Acad Dermatol 2013;69:578-82.)

Key words: fungal; irradiation; laser; neodymium:yttrium-aluminum-garnet; onychomycosis; Onychomycosis Severity Index; thermal.

Recently, lasers and light-based treatments, such as photodynamic therapy, have emerged as potential new treatment modalities. These treatments offer the advantage of having few contraindications, and minimal side effects.

We conducted a 4-part study designed to investigate a plausible mechanism of action of the neodymium:yttrium-aluminum-garnet (Nd:YAG) 1064-nm laser in onychomycosis. This type of laser was the first Food and Drug Administration–approved device for the cosmetic improvement of onychomycosis. We hypothesized that fungal organisms may be killed in vitro either by a thermal effect, or a direct lethal effect on viable fungi. The in vivo arm was designed to evaluate the efficacy and safety of the Nd:YAG 1064-nm laser (Laser Genesis, Cutera Inc, Brisbane, CA) on the treatment of mild, moderate, and severe toenail onychomycosis.

Abbreviations used:

CFU:	colony-forming unit
Nd:YAG:	neodymium:yttrium-aluminum-garnet
OSI:	Onychomycosis Severity Index

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METHODS

In vitro

Three common, but unrelated, nail pathogens were used for the in vitro portion of the study. *Trichophyton rubrum*, *Epidermophyton floccosum*, and *Scytalidium dimidiatum* were grown on agar slants. *S hyalinum* was selected as a representative nondermatophyte mold. Fungal elements were teased off the colony into sterile water to make a suspension of 0.4×10^4 to 5×10^4 colony-forming unit (CFU)/mL. Each fungal suspension was placed into 8 0.2-mL polymerase chain reaction tubes. The tubes were then placed in a heat block at 7 different heat and time exposures: 5 minutes at 45°C; 2, 5, 10, and 15 minutes at 50°C; and 2 and 5 minutes at 55°C. The heated samples were then plated on potato dextrose agar and incubated at 30°C. Controls from each of the 3 fungal dilutions were not heated and also plated on potato dextrose agar. Digital photographs of each Petri dish were taken daily from the fourth day postseeding through the ninth day postseeding. Efficacy of the thermal treatment was estimated by counting the number of CFU growing on the dish. Confluent growth was defined as: 400 CFU for *T rubrum*, 1000 CFU for *E floccosum*, and 4 CFU for *S dimidiatum*. For all manual colony counts, the examiner was blinded to the time and temperature parameters for each of these Petri dishes.

CAPSULE SUMMARY

- Laser treatment of onychomycosis is Food and Drug Administration approved as a device for improvement of onychomycosis.
- The mechanism of action of laser on fungus is not secondary to thermal heating or direct laser effect.
- Further in vitro and in vivo study is warranted to determine the effectiveness of laser in the treatment of onychomycosis.

In a second phase of the in vitro study, 5-μL suspensions of 0.4×10^4 to 5×10^4 CFU/mL fungal elements of *T rubrum* were dropped onto potato dextrose agar in a Petri dish and irradiated using a submillisecond Nd:YAG laser (Laser Genesis, Cutera Inc) at 1064 nm, with the parameters shown in the Table I. The laser-treated fungi were then incubated at 30°C. The control group was also dropped on potato dextrose agar and incubated. During laser irradiation, the temperature of the potato dextrose agar was measured. Digital photographs and fungal colony assessments were done daily from the fourth day posttreatment through the ninth day posttreatment. Efficacy of the laser treatment was determined based on the size of the colony in the Petri dish.

In the third phase of the in vitro portion of the study, *T rubrum* was grown confluently on potato dextrose agar until red pigment was visible. Plugs of the mycelium were then cut from the plate using 3-mm skin biopsy punches. A corresponding 3-mm punch biopsy specimen was removed from an uninoculated potato dextrose agar (PDA) plate and the mycelium punch placed into the vacant spot. The mycelium punches were then exposed to an assortment of laser irradiation parameters as shown in Table I. Control colonies did not undergo laser irradiation. Digital photographs and fungal colony assessments were done daily from the fourth day posttreatment through the ninth day posttreatment. Efficacy of the laser treatment was determined by the size of the colony in the Petri dish.

In vivo

The fourth part of the study was a 24-week pilot study of 10 patients conducted at the University of Alabama at Birmingham to evaluate the efficacy and safety of a 1064-nm Nd:YAG laser on the treatment of toenail onychomycosis. The study was approved by the University of Alabama at Birmingham Institutional Review Board. Patients enrolled were between the ages of 19 and 65 yeas, had clinically diagnosed distal lateral subungual onychomycosis of at least 1 great toenail, had culture-proven dermatophyte onychomycosis, and had at least 2 mm of healthy nail growth measured from the proximal nailfold. Exclusion criteria included nail disease other than onychomycosis, a history of trauma to the target nail, use of prescription topical antifungal agents within 3 months of study enrollment, use of systemic antifungal agents within 6 months of study enrollment, or use of topical over-the-counter antifungal agents within 1 month of study enrollment.

Subjects were treated with the submillisecond Nd:YAG laser at 1064-nm wavelength with a fluence

Table I. 1064-nm Neodymium:yttrium-aluminum-garnet *Trichophyton rubrum* irradiation parameters

Experiment	Spot size, mm	Fluence, J/cm^2	Pulse width, μs	Rep rate, Hz	No. of shots
A	5	20	300	7	27
B	5	5	100	10	103
C	3	50	300	10	11
D	3	15	100	8	35

Rep, Repetition.